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Award Number: DAMD17-01-1-0775

TITLE: Multifactorial assessment of depleted uranium neurotoxicity

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REPORT DATE: October 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2002	Annual (1 Oct 2001 - 30 Sep 2002)

4. TITLE AND SUBTITLE

Multifactorial assessment of depleted uranium neurotoxicity

5. FUNDING NUMBER
DAMD17-01-1-0775

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*
This is the first year of a three-year project to evaluate neurotoxicty related to depleted uranium (DU) administration and stress in rats. In this year we determined dose levels of DU to be employed in kinetic studies, selecting single intraperitoneal doses of 1 or 10 mg/kg. The kinetic studies were also done, showing regional brain (cerebral cortex, hippocampus, striatum, cerebellum) uranium concentrations of 4-18 ng/g 8 hours and 3-17 ng/g 24 hours after exposure to 1 mg uranium/kg. Concentrations tended to be highest in the cerebellum and hippocampus at 24 hours. Animals exposed to 10 mg/kg exhibited brain uranium concentrations of 10-20 ng/gm 24 hours after exposure, with no significant brain regional differences. Data for 7, 10 and 30 days post-dosing are pending. Animals that were swim-stressed at the time of exposure to uranium had significantly lower concentrations of this metal in their hippocampus and cerebellum than unstressed rats 24 hours after exposure. There was no significant difference in the serum concentrations of these animals. Renal tubular necrosis was seen in uranium-dosed rats, with subsequent regeneration. These studies establish parameters for the subsequent neurotoxicological investigations of this project

14. SUBJECT TERMS	15. NUMBER OF PAGES
depleted uranium, neurotoxicity	26
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

This is a three-year study on the neurotoxic potential of depleted uranium (DU) in laboratory rats. The effect of stress on DU kinetics and toxicity will also be evaluated. Previous studies with Gulf War veterans and experimental animals exposed to embedded DU suggest that neurotoxicity may result from DU exposure (McDiarmid et al. 2000; Pellmar et al., 1999). The current investigation is designed to assess the neurotoxic potential of acute and chronic exposure to DU and the contribution of stress to expression of DU neurotoxicity. All studies are being performed with adult male Sprague-Dawley rats. Acute exposures are being performed with uranyl acetate, while chronic exposures will utilize solid DU particles. The kinetics of DU in various brain regions is being determined by inductively coupled plasma-mass spectrometry (ICP-MS) analysis in cortex, hippocampus, striatum, and cerebellum between 8 hours and 30 days after a single DU administration. Neurotoxicity will be assessed with behavioral, morphological, and biochemical endpoints. Behavioral assessment of neurotoxicity will utilize Functional Observation Battery (FOB), motor activity, and tests of learning and memory (by passive avoidance). Biochemical analyses will include quantification of neurotransmitters, determination of receptor number, indicators of oxidative stress, and changes in gene expression. Biochemical determinations will be made in the same brain regions examined for DU kinetics. Morphological studies will employ perfusion-fixation, multilevel sampling of the nervous system and contemporary light microscopic procedures to allow detailed evaluation of any lesions. Stress will be applied to animals using forced swimming as described in previous Gulf War Illness studies. These studies will help define the neurotoxic potential of DU and by correlation with serum uranium, aid in identifying individuals at risk for DU neurotoxicity.

This is the annual report for year one of this three year study, the major activities of which involved determination and evaluation of temporal and regional DU kinetics in rat brain following a single exposure to soluble DU. In addition, studies of associated renal lesions were also performed, to determine their impact on the neurological effects.

Body

I. Tasks from Statement of Work and Accomplishments- This is a complex study on the neurotoxic potential of depleted uranium and its modulation by stress. What follows are tasks described in the approved Statement of Work (see Appendix), along with relevant research accomplishments for Year 1.

a. Task 1- Perform preliminary studies to identify appropriate doses of DU for kinetic studies.

Research Accomplishments for Task 1. Preliminary studies were conducted to determine doses of uranyl acetate to be used in kinetic and toxicity studies.

Male Sprague-Dawley rats (200-225 g) were given a single intraperitoneal injection of uranyl acetate dissolved in sterile saline. Doses of 0, 10, 30, and 100 mg of uranium/kg body weight (given as uranyl acetate) were tested (n = 3). Following injections, animals were housed in plastic metabolism cages to allow collection of urine. Twenty-four hours after uranium administration, rats were killed by carbon dioxide inhalation and serum, kidney, and brain were collected. Urine was also collected over the entire 24 hour period. At necropsy, gross evidence of renal toxicity was observed in the 30 and 100 mg/kg dose groups, including pale swollen kidneys with perirenal edema (often bloody). Little or no urine was produced by rats in the 100 mg/kg group.

Samples were digested in glass pressure tubes using nitric acid and hydrogen peroxide. Following digestion, samples were quantitatively diluted to 5 ml and analyzed by ICP-MS at the University of Florida. Results of these studies are presented in Figure 1 A-D.

There was dose dependent increase in all samples except for the 100 mg/kg urine samples. Because little or no urine was produced by these animals, it is likely that urine production stopped before large amounts of uranium were excreted by this pathway. Due to the overt kidney toxicity and very high urine uranium levels produced at doses above 10 mg/kg, the decision was made to use doses at or below 10 mg/kg. However, the poor sensitivity of the instrument available at the University of Florida made obtaining data at lower doses from specific brain regions problematic. For this reason, we contracted with the Analytical Section of the Southwest Hazardous Waste Program, Hazard Identification Core at the University of Arizona to perform ICP-MS analysis of future samples. Using this instrument, we were able to determine uranium content of all samples for control animals.

Therefore, we chose 1 and 10 mg uranium/ kg doses for kinetic studies. These doses should produce relevant concentrations in body fluids and measurable tissue uranium levels.

Conclusion- Based upon the above data, single exposures of 1 and 10 mg/kg of uranium (given intraperitoneally as uranyl acetate) were chosen for the kinetic studies.

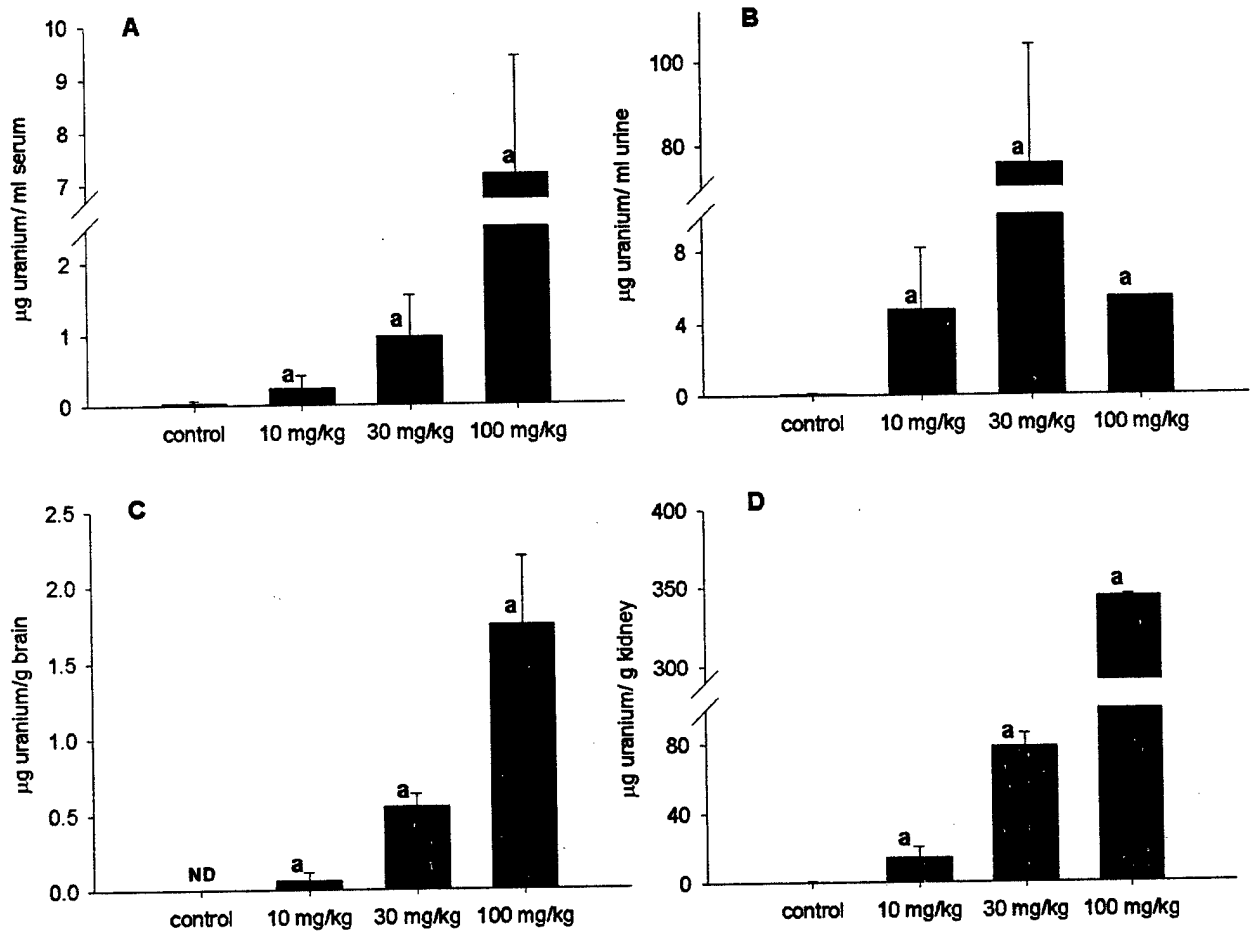


Figure 1. Uranium concentrations 24 hours after a single intraperitoneal injection of uranyl acetate. A. Uranium concentration in serum B. Uranium concentration in pooled 24 hour urine samples C. Uranium concentration in whole brain D. Uranium concentration in whole kidney. Values are mean +/- SD (n=3). [a] indicates values that are significantly different from control.

b. Task 2- Determine the concentration of DU in rat serum, brain cortex, hippocampus, striatum, and cerebellum at several times after DU exposure. The effect of stress on DU kinetics will also be determined.

Research Accomplisments for Task 2.

Study Design

Study design was a 2x2 array with 2 levels of stress (unstressed and stressed) and 2 levels of uranium (1 and 10 mg/kg), as indicated below. .

Saline control, no stress (n=5)	1 mg/kg uranium, no stress (n=20)	10 mg/kg uranium, no stress (n=20)
	1 mg/kg uranium, stress (n=20)	10 mg/kg uranium, stress (n=20)

The control group consisted of 5 unstressed animals that were injected with saline. Five male Sprague-Dawley rats (200-250 g) were used in each group. Stress was induced by 12-minute sessions of forced swimming. Each 12-minute session was composed of swimming for 4 minutes, resting for 4 minutes, and swimming for 4 minutes. Control animals were subjected to the same protocol, but were placed into empty tanks. This protocol was applied once daily for 5 days prior to uranium injection. One hour after the final swimming session, uranium was administered as an intraperitoneal injection of uranyl acetate in saline (1 ml/kg). Animals were returned to their home cages and allowed *ad libitum* access to food and water. Animals were killed by carbon dioxide inhalation at 8 hours, 24 hours, 7 days, and 30 days following uranium exposure. Five animals from each group were used at each timepoint. Saline controls were only sampled at 24 hours after injection.

Due to mortality that occurred on day 9 in animals in the 10 mg/kg groups (3 out of 10 animals), a decision was made to shorten the timecourse for this group to 10 days. On day 10 remaining animals were sacrificed, consequently there are no samples for day 30 after exposure for 10 mg/kg.

Determination of serum and regional brain uranium concentrations

Uranium content of serum and tissues was determined by ICP-MS analysis following nitric acid/peroxide digestion (for methods, see Appendix). At this time, all in-life animal work for this task has been completed. Data has been obtained and analyzed for the 8 and 24-hour timepoints. We are in the process of acquiring and completing analysis of data for 7, 10, and 30-day timepoints and anticipate that this will be

presented at the Force Health Protection Depleted Uranium Health Risks Assessment Projects Review in Albuquerque, NM, December, 2002. Results of these studies are described in the abstract by Barber and Kopplin (Abstract #1 in the Appendix) which has been submitted to the Society of Toxicology for presentation at its 2003 Annual Meeting.

The concentration of uranium in serum and selected brain regions 8 and 24 hours after exposure is presented in figure 2. The effect of stress on uranium concentrations is presented in figure 3.

Conclusions- Brain uranium concentrations of 4-18ng/g were observed 8 hours after intraperitoneal exposure to 1 mg uranium/kg. Concentrations tended to be highest in the hippocampus and striatum. 24 hours after exposure, brain uranium concentrations of 3-17 ng/g were observed with the highest concentrations in the hippocampus and cerebellum. Animals exposed to 10 mg uranium/kg exhibited brain uranium concentrations of 10-20 ng/g 24 hours after exposure, though there were no significant differences between brain regions.

Animals that were stressed at the time of exposure to 1 mg uranium/kg had significantly lower uranium concentrations in their hippocampus and cerebellum than unstressed animals 24 hours after exposure. There was no significant difference in the serum concentrations of these animals. Stressed animals in the 10 mg uranium/kg treatment group tended to have lower levels of uranium in the hippocampus and cerebellum with higher serum levels, but there was no significant difference.

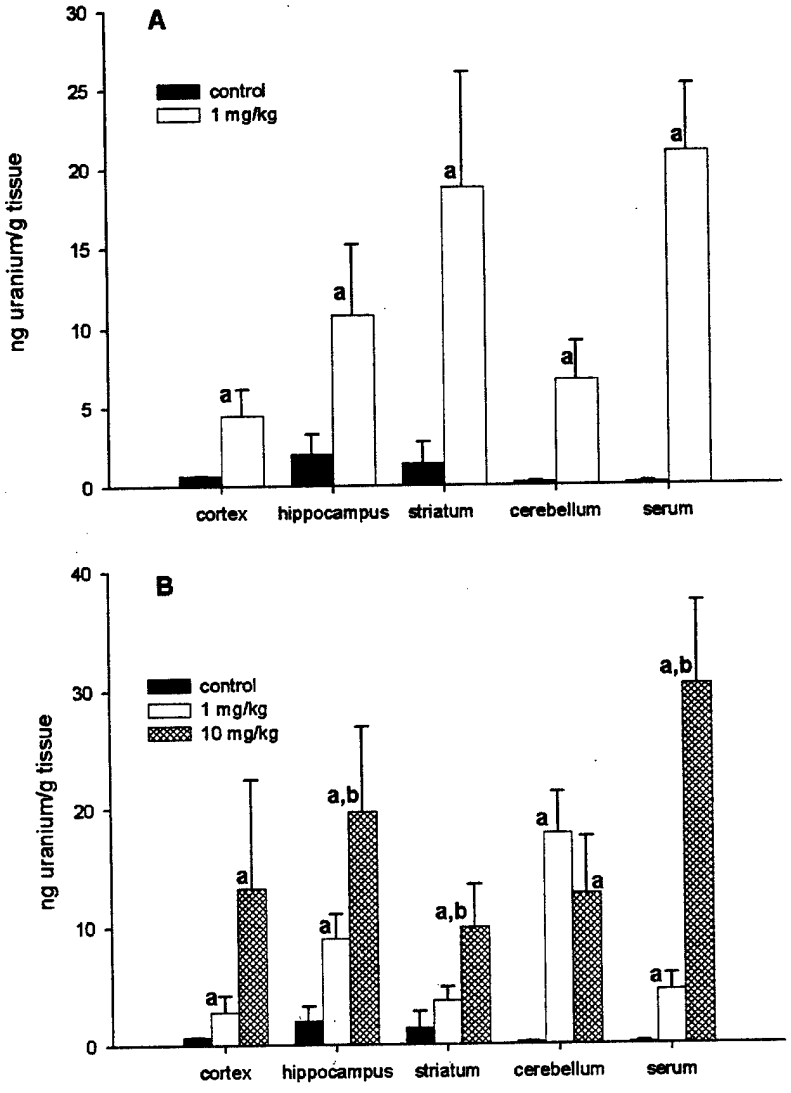


Figure 2. Uranium concentrations in rat serum and brain regions in unstressed animals. A. 8 hours after uranium administration Data from 10mg/kg treatment group is pending. B. 24 hours after uranium administration. Values are presented as mean +/- SD (n=5).
[a] indicates values significantly different from control ($P<0.05$).
[b] indicates values significantly different than 1 mg/kg treatment group ($P<0.05$).

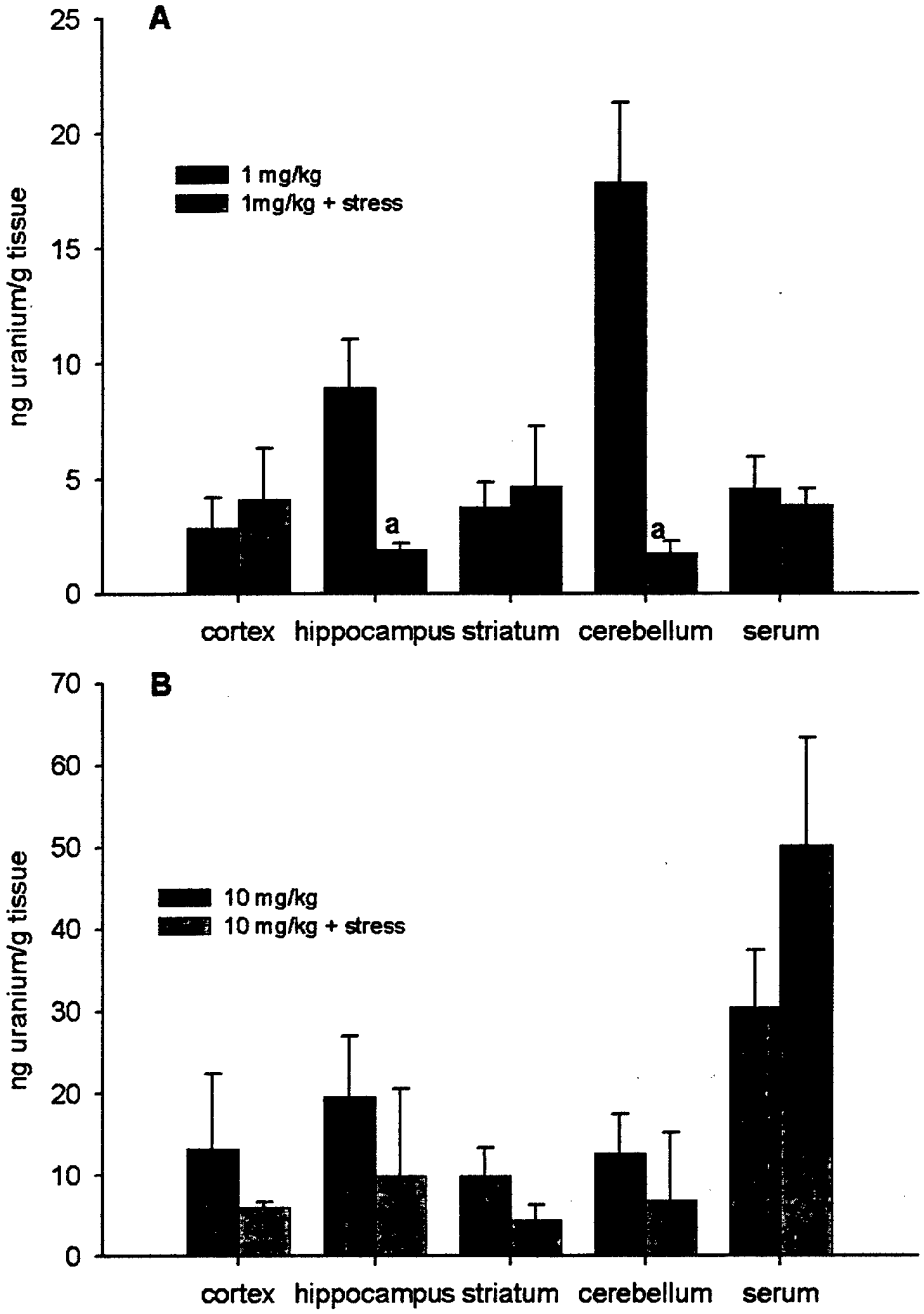


Figure 3. Effect of stress on tissue uranium concentrations 24 hours after uranium exposure.
A. Tissue uranium in stressed and unstressed rats treated with 1mg uranium/kg.
B. Tissue uranium in stressed and unstressed rats treated with 10 mg uranium/kg.
Values are presented as mean +/- SD (n=5). [a] indicates values that are significantly different from unstressed animals ($P<0.05$).

c. Task 3- Establish a 2x3 factorial treatment model to study the acute neurotoxicity of DU in the rat.

This study using neurobehavioral, neurochemical and neuropathological approaches will be initiated when data from the kinetic study (Task 2) has been analyzed. In an effort to have the best stress experimental system, we are in the process of performing a comparative study of stress models in rats. These compare repeated restraint, swimming, oral corticosterone (400µg/ml in the drinking water) and routine handling (the control group) with n = 10/group. Sacrifice is at 28 and 63 days, with changes in body and organ weight, clinical biochemistry, gastric ulceration, and blood levels of corticosterone, epinephrine and norepinephrine being used as measures of stress. The study is currently in progress, with final sacrifice of rats scheduled for November 12, 2002.

d. Task 4- Ancillary studies outside Statement of Work

Two studies regarding uranium renal toxicity have been performed in conjunction with the above kinetic studies. Results of these studies have been submitted to the Society of Toxicology for presentation at its 2003 Annual Meeting (Abstracts #2 and #3 in the Appendix).

Study 1. Renal pathology produced by single administrations of uranyl acetate.

Kidneys from the DU kinetic study (Task 2, above) were collected and fixed in 10% neutral buffered formalin and processed for histological evaluation. Sections stained with hematoxylin and eosin revealed the presence of prominent degeneration and cell death of epithelium lining the proximal tubules in a dose-related fashion in animals administered a single 1 or 10 mg/kg dose of uranium (Figure 4, see Pomeroy *et al.*, Appendix- Abstract 3). This was seen as early as 8 hours post-dosing, and was prominent at 24 hours. Both necrosis and apoptosis (seen with TUNEL stain) appeared to be present. Regeneration of tubular epithelium, more prominent with the lower dosage, was active by 168 hours post-dosing (Figure 4). This study was undertaken to assess the role renal toxicity might have on studies of acute uranium neurotoxicity (Task 3).

Study 2. Role of heat shock proteins in resistance to uranium-induced nephrotoxicity.

Experimental evidence suggests that prior exposure to uranyl compounds can reduce nephrotoxicity of subsequent uranium exposures. The mechanism of this protection is not clear but has been attributed to heat shock protein (Hsp) induced cytoprotection (Mizuno et al., 1997). The current study examined the relationship between stress proteins and acquired resistance to uranyl compounds (see Munson *et al.*, Appendix- Abstract 3). Male Sprague-Dawley

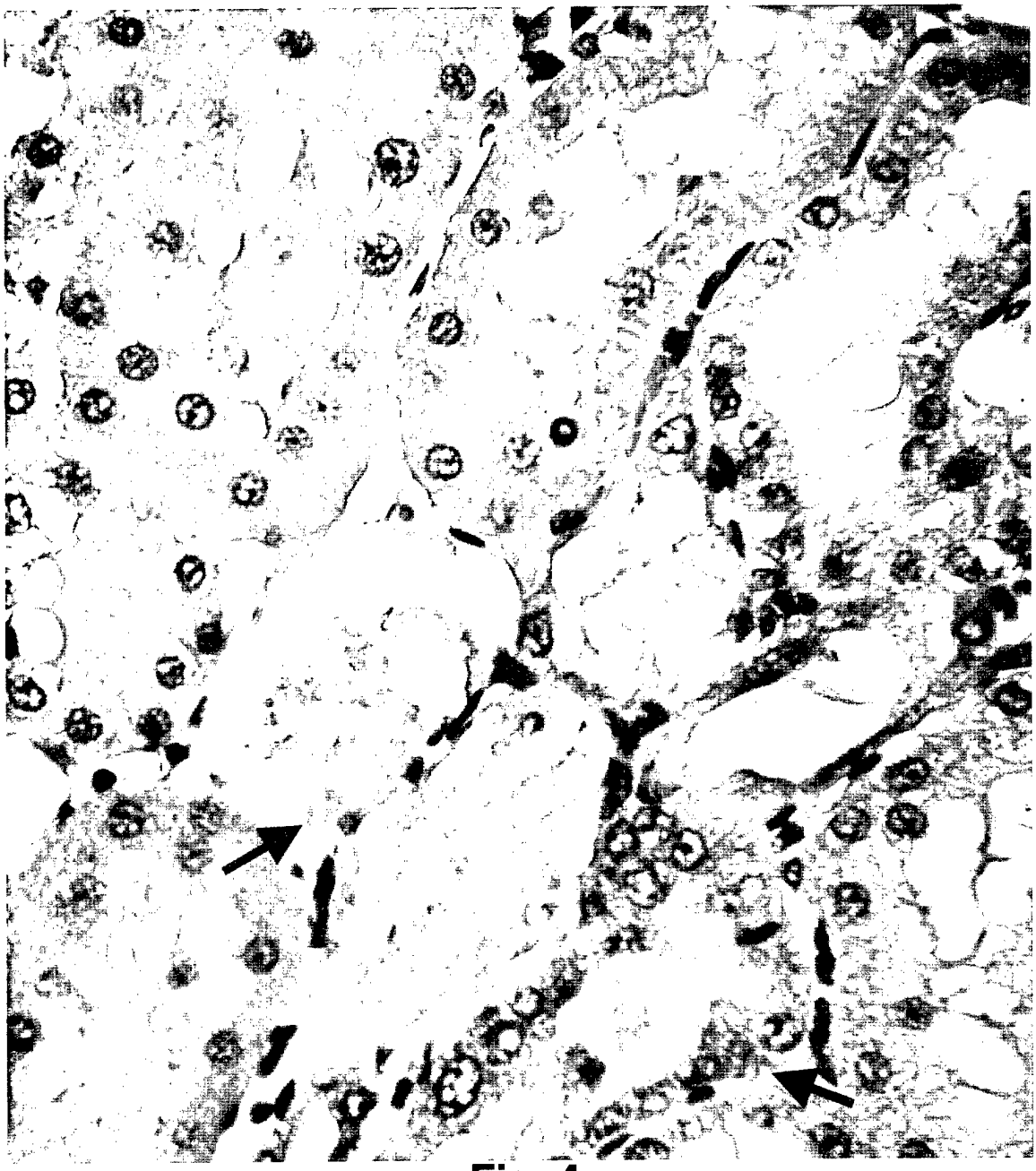
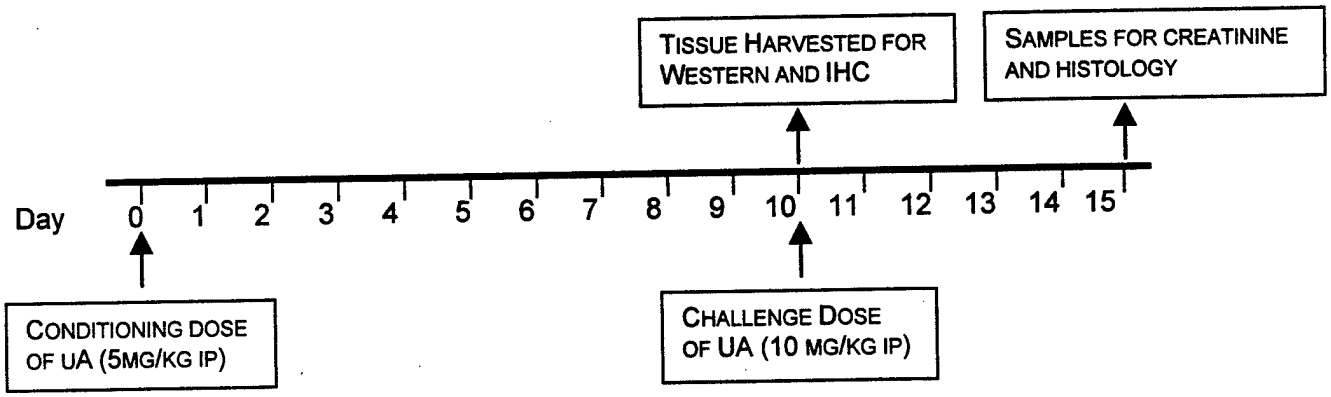


Fig. 4

rats were conditioned with 5 mg/kg uranyl acetate (UA) i.p. or saline (control) 10 days prior to challenge with 10 mg/kg i.p. UA. Samples of liver and kidney were taken at the time of the challenge dose to ascertain levels of Hsp present in each pre-treatment group. Five days after the second administration of UA, renal function (serum creatinine) and histopathological changes were evaluated.



Timeline of cytoprotection study. UA= uranyl acetate; IHC= immunohistochemistry

Based on histology and serum markers, a single acute dose of UA (10 mg/kg) produced acute proximal tubular necrosis. However, rats preconditioned with UA (5 mg/kg) exhibited improved kidney function (lower serum creatinine) and less tubular injury (figure 5). Kidney samples taken at the time of the second UA administration revealed strong induction of Hsp25, Hsp32 and Hsp70i, but not heat shock cognate 70 (constitutive form of hsp70, Hsc70), in preconditioned animals (figure 6). Immunohistochemical staining demonstrated that UA preconditioning upregulated Hsp70i and Hsp25 in the kidney, especially in what appeared to be regenerating tubular epithelium (figure 7). These observations demonstrate that several stress proteins are induced by UA administration and suggest that stress proteins confer resistance to UA-mediated nephrotoxicity by sparing proximal tubular epithelial cells, especially those undergoing active regeneration.

Conclusion- Pretreatment of rats with uranium decreased renal injury produced by subsequent exposure to uranium. This protective effect correlated with increased levels of Hsp 25, 32, and 70i, but not the constitutive form of hsp 70. Immunohistochemical staining suggests that regenerating tubular epithelium express high levels of heat shock proteins and may be responsible for observed uranium resistance.

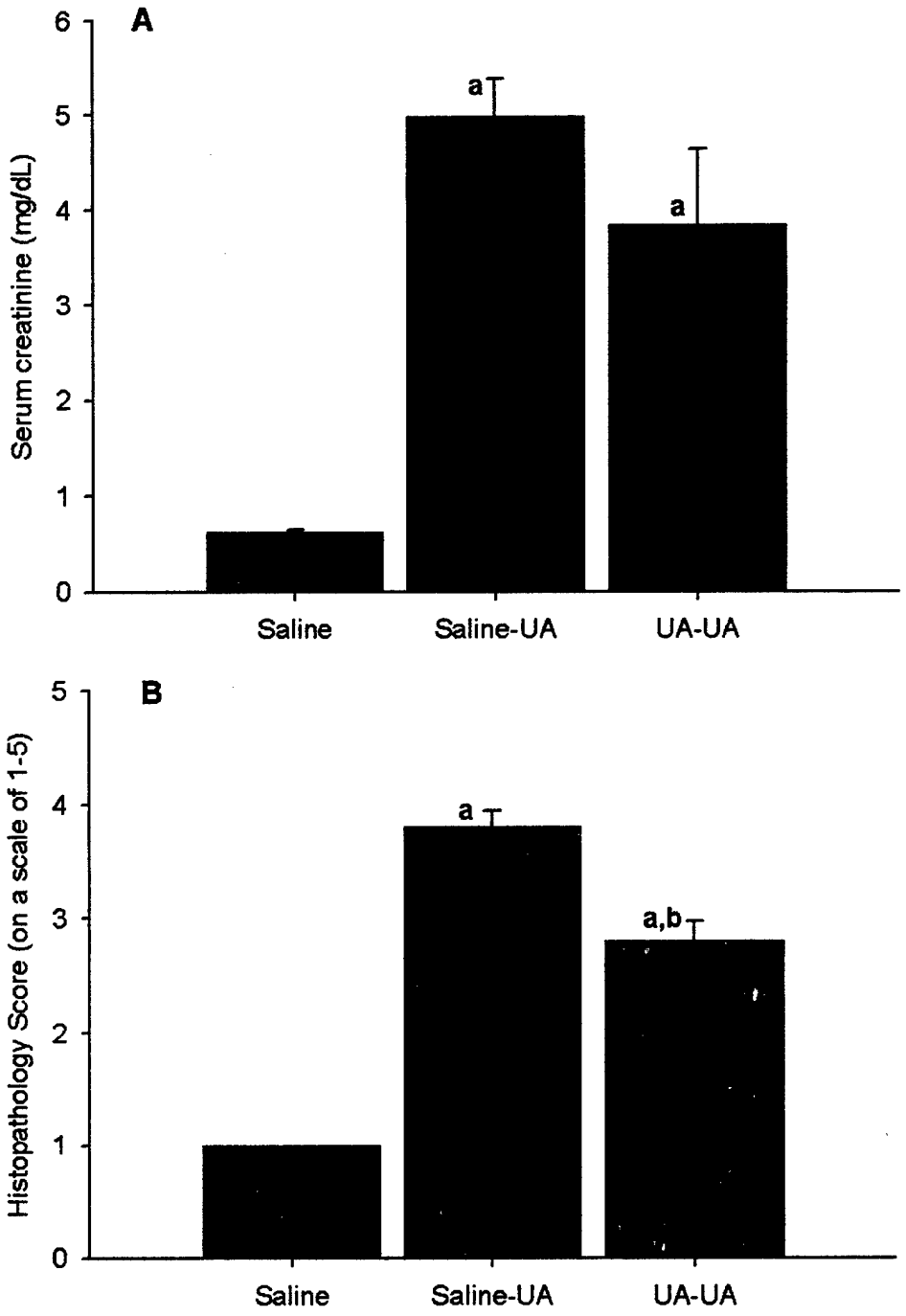


Figure 5. Protective effect of uranium onrenal toxicity from a subsequent dose of uranium. A. Serum creatinine levels (n=10) B. Histopathological scores of H&E stained kidney sections (n=10). Scores ranged from 1 (normal) to 5 (severely damaged). Values are presented as mean ± SD. [a] indicates values significantly different than control. b indicates values significantly different than saline pretreatment.

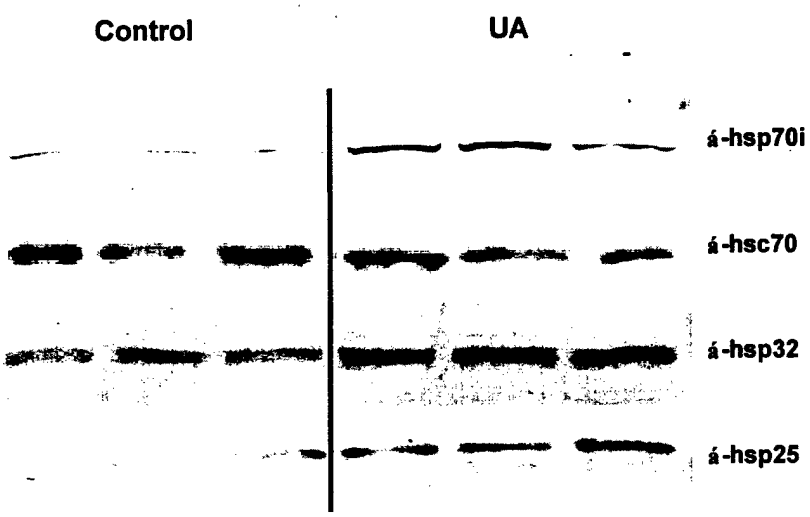


Figure 6. Western blot showing heat shock protein induction in kidneys from rats 10 days after treatment with 5 mg/kg uranyl acetate. While Hsp-70i, Hsp32, and Hsp25 exhibit induction, there is no apparent change in the level of constitutive heat shock protein 70 (Hsc70)

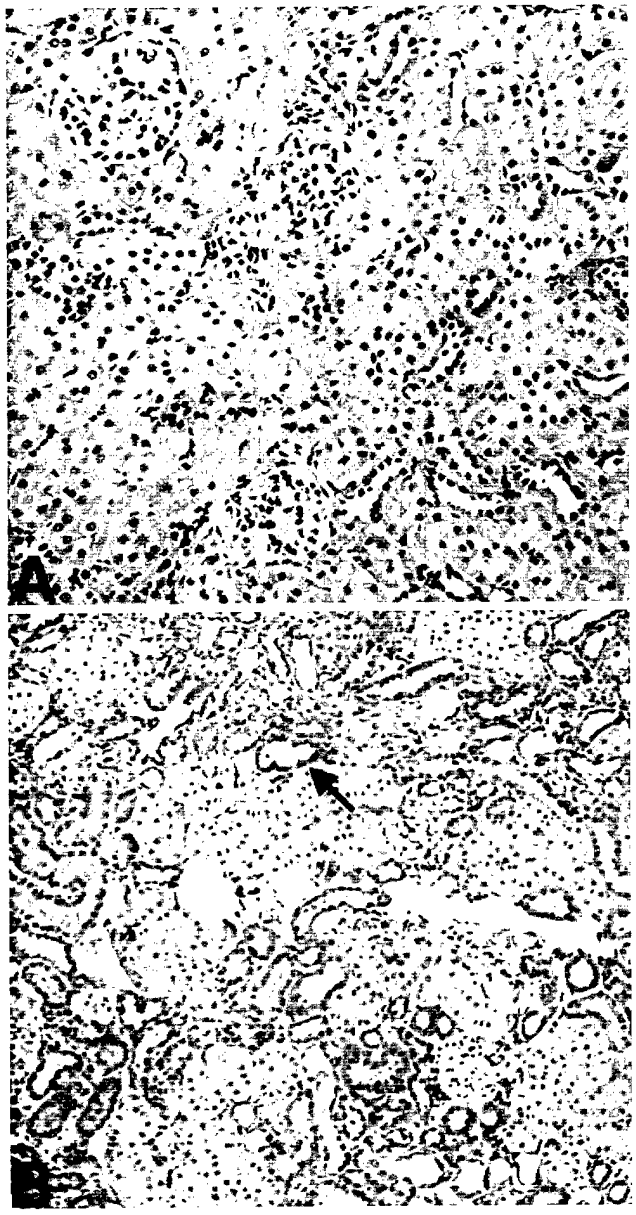


Fig.7

II. Problems Encountered, Solutions, and Recommended Approaches to Future Work.

a. Kinetic Studies- While we anticipated completion of kinetic studies by this point, several factors have contributed to their delay. Dr. Barber's relocation to the University of Florida in December of 2000, necessitated a subcontract with that institution. While the original award was made on October 1, 2001, administrative delays associated with the subcontract resulted in funds not being available to Dr. Barber at the University of Florida until March 26, 2002. This delayed the initiation of kinetic studies. The original technician working on this project resigned, but has been replaced. Initial uranium analyses were performed at the University of Florida, but low sensitivity of this instrument hampered the ability to successfully complete this study. As detailed above, we are now working with the Analytical Section of the Southwest Hazardous Waste Program, Hazard Identification Core at the University of Arizona to perform ICP-MS analysis.

b. Toxicity of 10 mg/kg dose of uranyl acetate- During the kinetic study, 30% of the rats treated with 10 mg uranium/kg as uranyl acetate died on day 9 which caused that study to be terminated on day 10. This may have an impact on our design of the acute toxicity study. However, since our renal pathology studies indicated that significant tubular epithelial regeneration was occurring at this time, therapy may mitigate this effect. Thus, we are currently working with Dr. Dru Forrester, a veterinary nephrologist at Virginia Tech, to determine an appropriate regimen to address this issue for the upcoming short-term neurotoxicity studies using uranyl acetate. This nephrotoxicity should not be a problem in subsequent long-term neurotoxicity studies using intramuscular DU particulate matter.

Key Research Accomplishments

- Preliminary studies to determine doses have been completed. The results of these studies indicated that doses of 1 and 10 mg uranium/kg would provide a group of animals with systemic distribution of uranium and produce detectable amounts of uranium in the brain.

- The kinetic study has been largely completed. Initial findings indicate that soluble uranium compounds rapidly enter the brain. In animals treated with 1 mg/kg, there were significant differences among brain regions, with hippocampus and cerebellum tending to have the highest levels of the regions examined. In animals under stress, there were significant reductions in uranium content in the hippocampus and cerebellum of animals treated with 1mg/kg 24 hours after exposure.

- Ancillary studies on renal pathology and the mechanism of cell death within the kidney were performed. Studies indicated that both doses of uranyl acetate produced injury to the tubular epithelium that led to both necrosis and apoptosis, with subsequent regeneration.

- Studies to determine the role of heat shock proteins in cytoprotection afforded by prior exposure to uranium were performed. These studies indicated that Hsp 25, 32, and 70_i were induced by prior exposure to uranium, especially in regenerating tubular epithelium.

Reportable Outcomes

Three abstracts were submitted for the 2003 Annual meeting of the Society of Toxicology.

Abstract 1. REGIONAL DISTRIBUTION OF URANIUM IN RAT BRAIN. D S Barber[1] and M J Kopplin[2]. [1] Center for Environmental and Human Toxicology. University of Florida, Gainesville, FL, USA; [2]Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ, USA.

Abstract 2. HEAT SHOCK PROTEINS AND URANIUM NEPHROTOXICITY. J W Munson[1], J K Tolson[1], B S Jortner[2], S M Roberts[1] and D S Barber[1] . [1]Center for Environmental and Human Toxicology. University of Florida, Gainesville, FL, USA; [2]Laboratory for Neurotoxicity Studies, VPI&SU, Blacksburg, VA, USA.

Abstract 3. URANIUM AND CELL DEATH IN THE RAT KIDNEY. M Pomeroy[1],B Jortner[1],M Ehrich[1],J Robertson[1], and D S Barber[2]. [1]Department of Biological Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, USA; [2]Department of Physiological Sciences, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, USA.

Conclusions

Serum, kidney, urine, and brain uranium concentrations were examined in rats treated with uranium (as uranyl acetate) at 10, 30, and 100 mg/kg. Based upon these, single intraperitoneal injections of 1 and 10 mg/kg of uranium (as uranyl acetate) were chosen for kinetic and short-term neurotoxicity studies. These should produce relevant concentrations of this metal in body fluids and tissues, including the brain.

Brain uranium concentrations of 4-18ng/g were observed 8 hours after intraperitoneal exposure to 1 mg uranium/kg. Concentrations tended to be highest in the hippocampus and striatum. Twenty-four hours after exposure, brain uranium concentrations of 3-17 ng/g were observed with the highest concentrations in the hippocampus and cerebellum. Animals exposed to 10 mg uranium/kg exhibited brain uranium concentrations of 10-20 ng/g 24 hours after exposure, though there were no significant differences between brain regions.

Animals that were stressed at the time of exposure to 1 mg uranium/kg had significantly lower uranium concentrations in their hippocampus and cerebellum than unstressed animals 24 hours after exposure. There was no significant difference in the serum concentrations of these animals. Stressed animals in the 10 mg uranium/kg treatment group tended to have lower levels of uranium in the hippocampus and cerebellum with higher serum levels, but there was no significant difference.

Significant renal injury was observed in animals from both treatment groups (1 and 10 mg/kg) and 30% of the animals treated with 10 mg/kg died 9 days after exposure. For this reason, further studies on the renal injury produced by these treatments were performed. Examination of the damaged kidneys revealed significant loss of tubular epithelial cells by necrosis and apoptosis. However, pronounced regeneration was present in both treatment groups by day 7 after exposure.

Pretreatment of rats with uranium decreased renal injury produced by subsequent exposure to uranium. This protective effect correlated with increased levels of hsp 25, 32, and 70i, but not the constitutive form of hsp 70. Immunohistochemical staining suggests that regenerating tubular epithelium express high levels of heat shock proteins and may be responsible for observed uranium resistance.

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Pellmar TC, Keyser DO, Emergy C, and Hogan JB. Electrophysiological changes in hippocampal slices isolated from rats embedded with depleted uranium fragments. NeuroToxicology 20:785-792. 1999.

Appendices-

Methods
Abstracts
Statement of Work
High quality prints of figures 4 and 7

Methods

1. Uranium analysis- Uranium analysis was performed by inductively coupled plasma-mass spectrometry (ICP-MS) on samples that had undergone nitric acid/peroxide digestion. Samples were placed in 15ml sealed glass pressure tubes with 0.5 ml of concentrated metal-free nitric acid (Optima, Fisher Scientific). Samples were heated to 140°C for 2 hours in a silicone oil bath, then 0.5ml of 30% hydrogen peroxide (Ultrex II, J.T. Baker) was added and samples heated at 110°C for a further 60 minutes. Samples were cooled, quantitatively transferred to acid-washed 5ml volumetric flasks, and brought to volume with deionized water (ElgaStat Maxima).
   The limit of quantitation of this method under normal conditions was 0.01 ppb. By using a lower range of standards, the limit of quantitation was improved to 0.002ppb to accurately determined uranium concentrations from samples with low levels of uranium. Recovery was determined from samples spiked with 0.1-10ppb uranium and determined to be 96-108%.

2. Pathology- Tissues (liver, kidney, heart) were immersion-fixed in 10% neutral buffered formalin, and them embedded in a mixture of paraffin and plastic polymers, sectioned at 5 µm thickness and stained with hematoxylin and eosin and with the TUNEL technique (for apoptosis).

3. Immunohistochemistry- To preserve antigenicity, samples were fixed with 10% buffered formalin for 4 hours then transferred to 70% ethanol for 24 hours. Staining was performed on paraffin embedded sections. Detection of proteins was accomplished with HistoRed.

4. Statistical Analysis- Data was analyzed by one- and two-way analysis of variance using Fisher's LSD post-hoc test for all pairwise comparisons. Mean values were considered significantly different if $P<0.05$. Statistical analysis was performed with SigmaStat 2.0 (Jandel Scientific, San Rafael, CA).

Abstract #1

REGIONAL DISTRIBUTION OF URANIUM IN RAT BRAIN. D S Barber[1] and M J Kopplin[2]. [1] Center for Environmental and Human Toxicology. University of Florida, Gainesville, FL, USA; [2]Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ, USA.

It has been suggested that depleted uranium exposure contributed to symptoms of Gulf War Illness. Previous studies have demonstrated that depleted uranium alters hippocampal neuron function and indicate that uranium causes neurotoxicity. However, it is not clear to what extent uranium accumulates in various brain regions, which is likely to determine the pattern of neurotoxicity. The distribution of brain uranium was quantified in male Sprague-Dawley rats that were treated with a single injection of 1 or 10 mg uranium/ kg as uranyl acetate by intraperitoneal injection. To determine if physiological stress has an impact on uranium distribution or accumulation, a subset of animals underwent periods of forced swimming for 5 days prior to uranium injection. The concentration of uranium in serum, hippocampus, striatum, cerebellum, and frontal cortex was determined by ICP-MS at 8 hours, 24 hours, 7 days and 30 days after exposure. Both doses of uranium increased uranium content of all brain regions tested. In vehicle controls, uranium concentrations in cerebellum, cortex, hippocampus, and striatum were 0.18, 0.66, 2.0, and 1.4 ng/g respectively. Twenty-four hours after administration of 1 mg uranium/kg, values for these tissues were 17.8, 2.8, 8.9, and 3.7 ng/g, respectively, while in animals given 10 mg uranium/kg, tissue concentrations were 12.7, 13.3, 19.7, 9.9 ng/g respectively. In both dose groups, stress tended to reduce brain concentrations without markedly affecting serum values. These studies demonstrate that soluble uranium rapidly enters the brain and exhibits regional distribution. (Supported by: U.S. Army Medical Research and Materiel Command DAMD17-01-1-0775. This abstract does not necessarily reflect the position or policy of the US Government.)

Abstract #2

HEAT SHOCK PROTEINS AND URANIUM NEPHROTOXICITY. J W Munson[1], J K Tolson[1], B S Jortner[2], S M Roberts[1] and D S Barber[1] . [1]Center for Environmental and Human Toxicology. University of Florida, Gainesville, FL, USA; [2]Laboratory for Neurotoxicity Studies, VPI&SU, Blacksburg, VA, USA.

Military use of depleted uranium has renewed interest in uranium toxicity. Experimental evidence suggests that prior exposure to uranyl compounds can reduce nephrotoxicity of subsequent uranium exposures. The mechanism of this protection is not clear but has been attributed to heat shock protein (Hsp) induced cytoprotection. This study examined the relationship between stress proteins and acquired resistance to uranyl compounds. Male Sprague-Dawley rats were conditioned with 5 mg/kg uranyl acetate i.p. (UA) or saline (control) 10 days prior to challenge with 10 mg/kg i.p. UA. Five days after the second administration of UA, renal function, pathology and Hsp expression were evaluated. Based on histology and serum markers, a single acute dose of UA (10 mg/kg) produced acute proximal tubular necrosis. However, rats preconditioned with UA (5 mg/kg) exhibited improved kidney function and pathology. Kidney samples taken at the time of the second UA administration revealed strong induction of Hsp25, Hsp32 and Hsp70i, but not Hsc70, in preconditioned animals. Immunohistochemical staining

demonstrated that UA preconditioning upregulated Hsp70i throughout the kidney while Hsp25 was highly localized in regenerating tubular epithelium. These observations demonstrate that several stress proteins are induced by UA administration and suggest that stress proteins confer resistance to UA-mediated nephrotoxicity by sparing proximal tubular epithelial cells, especially those undergoing active regeneration.

Abstract #3

URANIUM AND CELL DEATH IN THE RAT KIDNEY. M Pomeroy[1],B Jortner[1],M Ehrich[1],J Robertson[1], and D S Barber[2]. [1]Department of Biological Sciences and Pathobiology, Virginia Tech, Blacksburg, VA, USA; [2]Department of Physiological Sciences, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, USA.

We investigated the histopathological course of uranium-induced acute tubular necrosis in adult male Sprague-Dawley rats, focusing on pathogenic events. Rats were sacrificed 8 hours, 24 hours, and 168 hours following intraperitoneal administration of 0, 1 or 10 mg/kg of uranium given as uranyl acetate in saline. Mean total uranium concentration (in ng/ml, n = 3-5) in serum was as follows: 1 mg/kg dose- 20.9 at 8 hours, 4.5 at 24 hours; 10 mg/kg dose- 200 at 8 hours, 30.5 at 24 hours. Light microscopic studies revealed diffuse brush border loss and necrosis of renal proximal tubular epithelium, most marked in rats given 10 mg/kg, confirming earlier work documenting the toxic effect of uranium (Sanchez et al., Biol Trace Elem Res 84:139; Lim et al., Yonsei Med J 28:38). This lesion was notable at 8 hours post-dosing in the outer stripe of the medulla, and extended through the cortex by 24 hours, best seen in rats given 10 mg/kg. We have demonstrated that apoptosis is a feature of this event, most prominent at 24 hours post-dosing, using the TUNEL assay (1:25) (TUNEL Label, Roche Diagnostics). Glomeruli appeared intact. By 168 hours, proximal tubular epithelial regeneration was evident in the cortex and outer medullary stripe, more prominent in the 1 mg/kg dosage group. This was characterized by cells having basophilic cytoplasm and not infrequently, mitotic figures. We demonstrate that apoptotic events occur during uranium-induced renal tubular injury and that regeneration is a prominent sequel to such a heavy metal effect. (Supported by: U.S. Army Medical Research and Materiel Command DAMD17-01-1-0775. This abstract does not necessarily reflect the position or policy of the US Government.)

STATEMENT OF WORK

The proposed work will use the male Sprague Dawley rat to characterize the kinetics and toxicity of depleted uranium (DU) in the brain. The ability of stress to affect disposition and toxicity of DU will also be examined, as stress can alter the permeability of the blood-brain barrier and enhance neurodegeneration (Friedman *et al.*, 1996; Sinton *et al.*, 2000; McEwen, 1999). The studies would examine neurotoxicity of DU after acute and long-term exposure by using detailed morphological, behavioral, and biochemical methods. Effects of DU exposure on neurotransmitters and their receptors will be examined with extension to examination of oxidative stress and gene expression likely.

The *experimental design* will be a split plot design (SPD). The whole plot will be a factorial array in a randomized complete block design (RCBD). The treatments are stress (2 levels- stressed and unstressed) and DU dose (2-4 levels including negative controls). The blocking factor is rat and the sub-plot is brain region (4 levels). For kinetic studies, a 2x2 design will be used (2 doses of DU and 2 levels of stress [stressed or unstressed]). The stressor will be applied once daily for at least 5 days prior to DU exposure. DU in cortex, hippocampus, caudate-putamen, and cerebellum, as well as blood and urine, will be determined at several times after DU exposure (e.g., 8 hours, 1 day, 7 days, and 30 days). For acute toxicity studies, a 2x3 design will be used, consisting of 2 levels of stress (stressed and unstressed) and 3 levels of DU (control, low, and high). At several times after exposure (e.g., 1, 7 and 30 days), brain samples will be analyzed for neurotransmitter levels, receptor numbers, and oxidative stress. At later time periods (e.g., 7 and 30 days), samples will be taken for morphological analysis. For acute toxicity studies, behavioral testing will be performed before dosing and weekly thereafter. For long-term toxicity studies, a 2x4 design will be used with 2 levels of stress (stressed and unstressed) and 4 levels of DU (tantalum negative control, low, medium, and high concentrations of DU particles). Stress will be induced by applying the stressor daily for 14 days during the study. Animals will be exposed to DU for 6 months. Behavioral testing will be performed before dosing and every 3 weeks thereafter. After 6 months, samples will be taken for morphological and biochemical analysis. All determinations will be made on 3-5 separate samples.

The study will be conducted at two institutions, Virginia Tech (investigators B. Jortner [principal investigator and neuropathologist] and M. Ehrich [neurotoxicologist]) and the University of Florida (investigator D. Barber [heavy metal toxicologist]), using the following plan.

Months 1-4:
Preparations for study (both institutions) and preliminary studies to identify appropriate doses (University of Florida). Consultation on doses (Virginia Tech).
Months 5-9 (kinetics study):
Kinetics of DU (as uranyl acetate) in the brain and interaction of stress studied using 4 treatments (low DU, high DU, low DU + stress, and high DU + stress) at 4 times (e.g. 8 hours, 1 day, 7 days, and 30 days) after a single injection of DU (University of Florida). Data analysis (both institutions). Technician training and preparation for acute study (Virginia Tech).
Months 10-16 (acute toxicity study)
Toxicity of a single intramuscular injection of DU (as uranyl acetate) determined using 6 treatments (vehicle control, low DU, high DU, control + stress, low DU + stress, and high DU + stress) at 3 times (e.g. 1, 7, and 30 days after injection). In-life study, neurobehavioral studies, neuropathology (Virginia Tech). Post-sacrifice biochemistry, data analysis (both institutions).

Months 17-27 (chronic toxicity study)
Toxicity of intramuscular injection of DU particles determined using 8 treatments (tantalum control, low DU, medium DU, high DU, tantalum + stress, low DU + stress, medium DU + stress, and high DU + stress) at 6 months after initiation of DU exposure. In-life study, neurobehavioral studies, neuropathology (Virginia Tech). Post-sacrifice biochemistry, data analysis (both institutions).

Months 28-36 (follow up study):
Effect of DU exposure on gene expression determined using a single dose and time from the above studies. Alteration of gene expression will be determined using material such as the U34 Rat Neurobiology Array produced by Affymetrix (University of Florida). Completion of biochemical (both institutions) and neuropathological (Virginia Tech) studies. Assembly of data and preparation of final report (both institutions).